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281

EDVO-Kit #

Principles & Practice of Diffusion & Osmosis

Storage:

Store entire experiment
at room temperature.

EXPERIMENT OBJECTIVE

The objective of this experiment is to develop an understanding of the molecular basis of diffusion and osmosis and its physiological importance. Students will analyze how solute size and concentration affect diffusion across semi-permeable membranes and how these processes affect water potential. Students will also calculate water potential of plant cells.

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This experiment is designed for 10 lab groups

Experiment Components

Contents

- A Orange Indicator Dye, low molecular weight
 - B Blue/green dye, high molecular weight
 - C 1M Sucrose and 1M NaCl
 - D Powdered sucrose
 - E NaCl
- Dialysis tubing

Store entire experiment at room temperature.

Requirements

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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- Beakers* (300 to 400ml)
 - 20 for Part A
 - 50 for Part B
 - 16 for Part C
- 1ml, 5ml, and 10ml pipets
- Graph paper
- Scales
- Distilled or deionized water
- Thermometers (10)
- Potatoes (4)
- Cork borers, or small kitchen knives
- Onion
- Microscope
- Slides
- Coverslips

**Beakers can be substituted with clear disposable plastic cups.*

Principles & Practice of Diffusion and Osmosis

DIFFUSION

Diffusion is the net flow of molecules from a region of high concentration to a region of low concentration. This difference in concentration of a substance across space is called a **concentration gradient**. Diffusion is due to the random movement of particles. This phenomenon

was first observed by Robert Brown in 1827 and is called **Brownian movement**. All objects in motion have **kinetic energy**, or energy of motion. Particles of matter move in straight lines until they collide with other particles. After colliding, the particles rebound, move off in straight lines until the next collision. There is no loss of energy. Diffusion will continue until there is no concentration gradient (**Figure 1**).

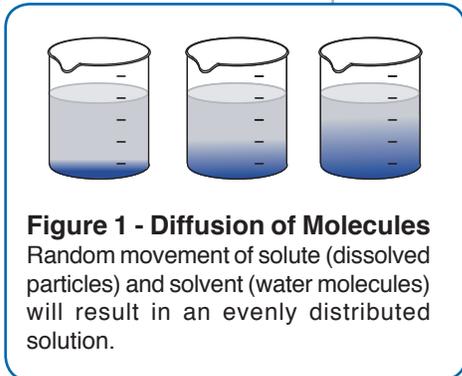


Figure 1 - Diffusion of Molecules
Random movement of solute (dissolved particles) and solvent (water molecules) will result in an evenly distributed solution.

In diffusion, molecules move randomly colliding with one another until they become evenly distributed. For example, if one puts a teaspoon of a purple dye, potassium permanganate, into a beaker of water, then the dye molecules, or **solute** (dissolved molecules), will collide randomly with the water molecules, or **solvent**. These random collisions within the **solution** will scatter the molecules of solute and solvent until they are evenly mixed. However, the molecules still

continue to collide with each other and move about randomly. At this point, there is no overall change in concentration. This condition is known as **dynamic equilibrium**. A system is most stable when it has reached equilibrium. A system will tend to go to equilibrium (lowest, accessible energy state) in the absence of added energy (**Figure 2**).

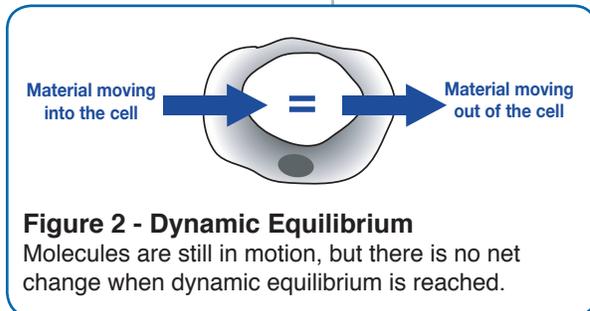


Figure 2 - Dynamic Equilibrium
Molecules are still in motion, but there is no net change when dynamic equilibrium is reached.

OSMOSIS

Osmosis is a special type of diffusion. It is the diffusion of **water** molecules across a semi-permeable membrane (a membrane that allows for the diffusion of certain solutes and water) from

an area of higher water concentration to one of lower water concentration. For example, if a 1 M aqueous starch solution is separated from a .5 M aqueous starch solution by a semi-permeable membrane, then water molecules will move from the .5 M aqueous starch solution (higher water molecule concentration) toward the more concentrated 1M starch solution (lower water molecule concentration) until an **equilibrium** of water molecules exists between the two solutions. Since the semi-permeable membrane did not allow for the passage of starch molecules, the 1M-starch solution will gain in volume as the water moves in (**Figure 3**).

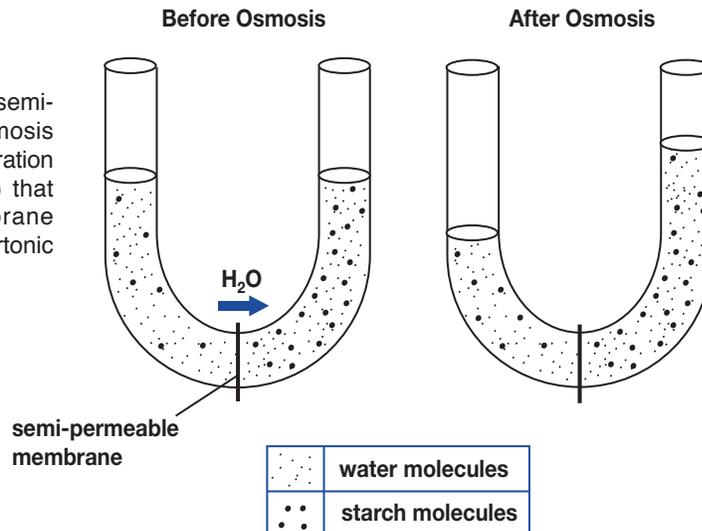


Principles & Practice of Diffusion and Osmosis

Background Information

Figure 3 - Osmosis

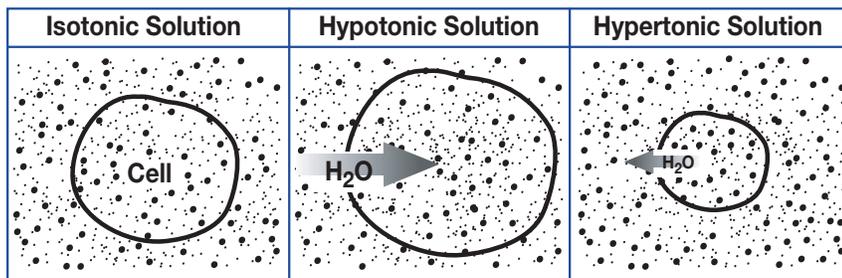
Water molecules move across a semi-permeable membrane during osmosis when one side has a higher concentration of a dissolved substance (solute) that cannot pass through the membrane (from hypotonic solution to a hypertonic solution).



All unicellular and multicellular organisms are surrounded by water solutions. A solution in which the concentration of dissolved substances or solutes is the same as the concentration inside the cell is an **isotonic solution**.

It also means that the concentration of water is the same as inside the cell. The cell is in dynamic equilibrium in an isotonic solution. These living cells will not be damaged by a gain or loss of water.

A solution in which the concentration of solutes is lower than the concentration inside the cell is called a **hypotonic solution**. However, the water concentration is higher inside the cell. A cell placed in a **hypotonic** solution will gain water by osmosis and swell in size. This results in an internal pressure. An animal cell, lacking a rigid cell wall, will swell and may **lyse**, or burst, in a hypotonic solution. A plant



| | |
|--|---------------------|
| | water molecules |
| | dissolved materials |

Figure 4 - Solution concentration's effect on cells.

The amount of water entering and leaving cells placed in isotonic solutions is the same. Cells will remain the same size and shape. The cells placed in hypotonic solutions will gain water and swell, while those placed in hypertonic solutions will lose water and shrink.



Principles & Practice of Diffusion and Osmosis

cell, having a rigid cell wall will be able to resist the pressure. This increase within a plant cell is known as **turgor pressure**. Turgor pressure gives support and shape to plant cells (**Figure 4**).

A **hypertonic solution** is a solution in which the concentration of solutes is higher than the concentration inside the cell. Therefore, the water concentration is lower than inside the cell. Animal cells placed into a hypertonic solution will lose water and shrivel up due to the decreased pressure inside the cell. A plant cell placed in a hypertonic solution will lose water from its large central vacuole. The plasma membrane and cytoplasm will shrink away from the cell wall, The end result is the loss of water and a decrease in turgor pressure and is known as **plasmolysis**. This is commonly known as wilting.

PASSIVE AND ACTIVE TRANSPORT

The plasma membrane is a highly selective barrier consisting of two layers of lipid. Embedded in these layers are a wide variety of proteins, glycoproteins, and glycolipids. The membrane components are always in a dynamic state of flux, which may create transient pores. Solutes may move through the membrane by either **passive** or **active transport**. **Passive transport** occurs when a solute molecule diffuses down a concentration gradient. There is no expenditure of energy. No ATP is used. Those molecules that are less polar (more lipid soluble) will generally penetrate the membrane more rapidly than polar molecules (more water soluble). However, small polar molecules such as water pass directly through the membrane pores (**Figure 5**).

Passive diffusion of larger molecules possessing high polarity and charge such as sugars and amino acids enter the cell via mediated transport mechanisms. The process known as **facilitated diffusion** uses a carrier protein in the plasma membrane to facilitate the speed of movement of large molecules from a region of high concentration to low concentration. A carrier protein selectively binds to a solute molecule on one side of the membrane, undergoes a conformational change, and releases the solute molecule on the other side of the membrane. Sugar molecules are transported in this manner. Other transport proteins provide passageways by which selective molecules may enter and leave a cell. Most of these dissolved materials would not be able to diffuse through the lipid bilayer (**Figure 5**).



Principles & Practice of Diffusion and Osmosis

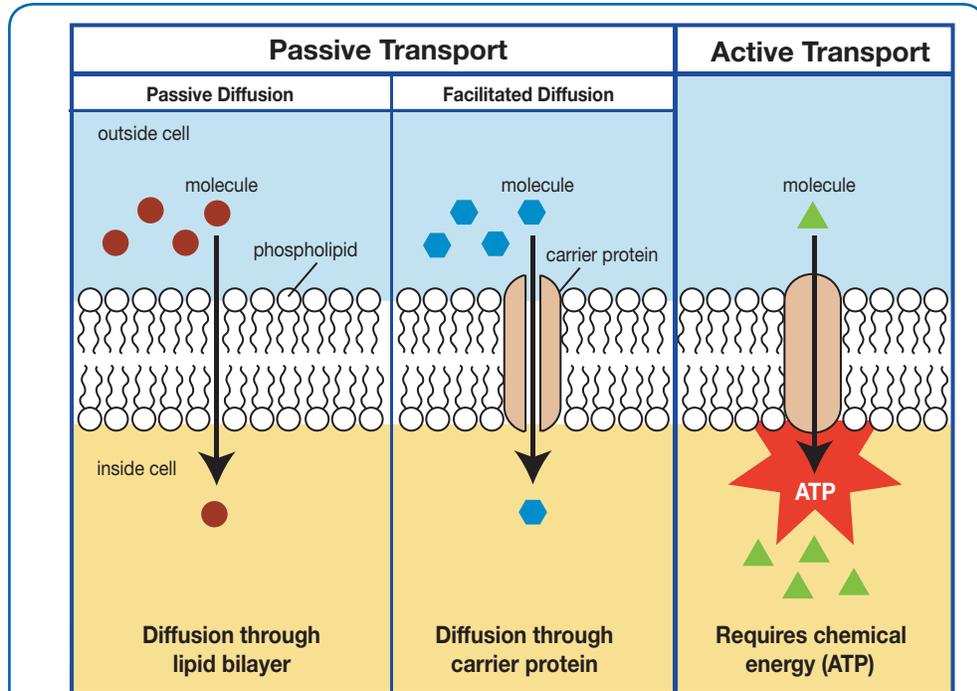


Figure 5 - Comparison of Passive Diffusion, Facilitated Diffusion, and Active Transport

In passive diffusion, hydrophobic molecules and small, uncharged molecules move down their concentration gradient directly across the membrane without the expenditure of energy. In facilitated diffusion, hydrophobic molecules diffuse through a transport protein down their concentration gradient across the membrane. Active transport move molecules up against their concentration gradient by mean of a transport protein. This requires the expenditure of ATP for energy.

Active transport occurs when a solute molecule is moved across a membrane against the concentration gradient by the utilization of chemical energy, or ATP. Active transport can create intracellular concentrations of sugars and amino acids 2 to 50 times higher than extracellular concentrations. A proton pump uses ATP to pump hydrogen ions out of the cell and produce a proton gradient with a higher concentration outside of the cell.

Principles & Practice of Diffusion and Osmosis

The net uptake or loss of water by the cell depends on which component, the extracellular or cellular fluids, has the **highest water potential**. Water potential is abbreviated by the Greek letter **psi (Ψ)**. Water potential is affected by two physical factors, that is solute concentration (solute potential, Ψ_s) and applied pressure component (pressure potential, Ψ_p). Remember water always moves across a membrane from the solution of **higher** water potential to one with **lower** water potential. The effects of pressure and solute concentration on water potential are represented by this equation:

$$\begin{array}{rcl} \Psi & = & \Psi_p \quad \quad \quad \Psi_s \\ \text{Water} & = & \text{Pressure} \quad \quad \quad + \quad \text{Solute} \\ \text{Potential} & & \text{potential} \quad \quad \quad \text{potential} \end{array}$$

The addition of solutes results in a higher osmotic potential and a decrease in the water potential of the system into the negative range. An increase in pressure raises the water potential of the system into the positive range. Water movement is directly proportional to the pressure on a system. The lower the water potential of a solution, the greater the tendency of water molecules to move into it by osmosis. For example, if potato cells are placed in pure water there will be a net influx of water into the cells, since pure water has a water potential of zero and the water potential in the cell is lower or more negative due to the cytoplasmic solutes. The potato cells will swell and gain in mass. There will be an increase in turgor pressure. When the water potential of the cell equals the water potential of the pure water outside the cell, a dynamic equilibrium is reached and there will be no net water movement.

Likewise, if potato cells are placed in sucrose solutions where the water potential of the cells are higher than the water potential of the sucrose solutions, there will be a flow of water out of the cells. The cells will shrink and lose mass. Therefore, the addition of sucrose to the water outside the potato cells, results in a decrease in the water potential of the solutions surrounding the cells. One can add an amount of sugar to the water, so that the water potential outside the cell is the same as the water potential inside the cell. There will be no net movement of water. However because the water potential inside the cell results from the combination of both pressure potential and solute potential, the solute concentrations inside and outside the cell will not be equal. If one continues to add sugar to the solution outside the cell, water will leave the cells as it moves from an area of higher water potential to an area of lower water potential. Plasmolysis of the cells will result.



Principles & Practice of Diffusion and Osmosis

WATER POTENTIAL

Water potential can be calculated by first calculating the solute potential of a sucrose solution using the following formula:

$$\Psi_s = -iCRT$$

i = Ionization constant (since sucrose does not ionize in water, it is 1.0).

C = Molar concentration of solute

R = Pressure constant (R = 0.0831 liter bars/mole K).

T = Temperature K (°C of solution + 273)

The water potential of the solution can be calculated by knowing the solute potential of the solution and knowing that the pressure potential of the solution is zero. The water potential will be equal to the solute potential of the solution.

$$\Psi = \Psi_s$$

In **Part A**, dialysis experiments will be done with two dyes of different molecular weights to visually demonstrate the size selectivity of membranes. The experiments will also demonstrate changes in the equilibrium of the diffusible dye as it is removed from the system.

Part B of the experiment will approximate an osmotic system by demonstrating net flow of water through a dialysis membrane.

Part C involves the calculation of potato cell water potential from experimental data.

Part D demonstrates the effect a hypertonic solution has on plant cells.

Experiment Overview

EXPERIMENT OBJECTIVE

The purpose of this experiment is to understand enzyme catalysis. The specific student objectives are:

1. To understand by investigation the mechanisms and physiological importance of diffusion and osmosis.
2. To understand how solute size and concentration gradients affect diffusion across semi-permeable membranes.
3. To understand the concept of water potential and how it is affected by solute concentration and pressure potential.
4. To understand how plant cells respond to high solute concentration solutions (hypertonic solutions) and relate result in terms of plasmolysis.



LABORATORY SAFETY

Gloves and safety goggles should be worn routinely as good laboratory practice.



PART A. Diffusion and Dialysis

Dialysis membranes are made of purified cellulose containing microscopic pores. The pore size is controlled during manufacture. The pore size determines the membrane's permeability to solutes of different sizes. Increasing size generally corresponds to increasing molecular weight when molecules have similar shapes. The dialysis tubing being used in this experiment has a molecular cut off of approximately 10,000, which means that molecules having molecular weights greater than 10,000 cannot penetrate the membrane. The orange dye has a molecular weight of about 300 and the blue/green dye contains a blue and a green dye, one of which has a molecular weight in excess of 100,000.

1. Tie a knot at one end of each piece of dialysis tubing. Start approximately one inch from the end. **DO NOT TIE THE KNOT TOO TIGHTLY**, otherwise tubing may tear or puncture. Keep tubing moist but avoid having too much water inside.
2. Fill each of 2 beakers (300 to 400ml in size) with 250ml of distilled water.
3. Add 1ml distilled water to an empty test tube with 1ml pipet.
4. Add 1ml of blue/green dye to the water in the test tube (Step 3) with the 1ml pipet. Mix.
5. Transfer all the 2ml of diluted blue/green dye with a pipet to one of the dialysis tubes.
6. Tie a knot at the open end of the tubing as instructed in Step 1.
7. Place the tubing in one of the beakers of water.
8. Transfer 1ml of the blue/green dye to the test tube containing the orange dye with the 1ml pipet. Mix. Note the color.
9. Transfer all the 2ml of mixed dye to the other dialysis tubing with a pipet and tie the open end as instructed in Step 1.
10. Place the tubing in the second beaker of water. Occasionally mix by stirring the water in the beakers with a stirring rod or by swirling. Note any changes.
11. After 30 minutes briefly stir the beakers. Note any color changes.
12. Remove the dialysis tubing containing the dye mixture from the beaker and pour out the water.
13. Fill the beaker with 250ml of fresh distilled water and put the tubing back in the beaker.

Experiment Procedure

PART A. Diffusion and Dialysis

14. Do Step 15 during your next lab period (next day if possible).
15. Remove the dialysis tubing containing the mixed dyes from the beaker and pour out the water.
16. Fill the beaker with 250ml of distilled water and put the dialysis tubing back into the beaker.
17. During your next lab period remove the tubing and observe the color. Compare with the tubing that only contained the blue/green dye.

PART B. Osmosis

Osmosis is the net flow of water across a semi-permeable membrane due to changes in solute concentrations. Increases in solute concentrations decrease the concentration of water. Water diffuses from a region of higher concentration to a region of lower concentration. In this experiment, solutions containing different concentrations of sucrose and salt will be transferred to dialysis tubing which will then be placed in distilled water. This arrangement behaves like an osmotic system. It is not a true osmotic system because the sucrose and the salt also penetrate the membrane. However, the dialysis tubing will only be in the water bath for 30 minutes. As this is not long enough for the system to come to equilibrium, a net change in water distribution should be observed.

1. Tie a knot at one end of each of 5 pieces of dialysis tubing. Start to tie the knot approximately 1 inch from the end. **DO NOT TIE THE KNOT TOO TIGHTLY**, otherwise the tubing may rip or puncture as you pull.
2. Consecutively label 5 dry beakers (300 to 400ml) 0 M, 0.25 M, 0.5 M, 0.75 M and 1 M.
3. Transfer 5ml of distilled water to a piece of tubing with a 5ml pipet and tie the open end as described in Step 1. Place the tubing in the beaker labeled 0 M.
4. Transfer 5ml of 0.25 M sucrose-salt solution to a piece of tubing and tie the open end as described. Place the tubing in the beaker labeled 0.25 M.
5. Transfer 5ml of 0.5 M sucrose-salt solution to a piece of tubing and tie the open end. Place tubing in the beaker labeled 0.5 M.



PART B. Osmosis

6. Transfer 5ml of 0.75 M sucrose-salt solution to a piece of tubing and tie the open end. Place in beaker labeled 0.75 M.
7. Transfer 5ml of 1 M sucrose-salt solution to a piece of tubing and tie the open end. Place tubing in the beaker labeled 1 M.
8. Briefly blot each piece of tubing with paper towel to dry. Weigh each piece to the nearest tenth of a gram. Record the mass as the initial mass in Table B1.
9. Immerse the dialysis tubing by filling each beaker with 250ml of distilled water.
10. After 30 minutes, remove the tubing and blot dry with paper towel. Weigh each piece and record the mass as final mass in Table B1.
11. Determine percent change between initial mass and final mass:

$$\frac{\text{FINAL MASS} - \text{INITIAL MASS}}{\text{INITIAL MASS}} \times 100 = \% \text{ CHANGE}$$

Enter these values in Table B1 under %. Do not be concerned if the tubing containing the distilled water has a slightly smaller or larger final mass. This is due to experimental error.

12. Graph the percent change on the Y-axis versus the molarity on the X-axis.

PART B. Analysis of Results: Osmosis

TABLE B-1: DIALYSIS BAG RESULTS - INDIVIDUAL DATA

| Molarity of Dialysis Bag | Initial Mass | Final Mass | Mass Difference | % Change in Mass |
|--------------------------|--------------|------------|-----------------|------------------|
| 0.00 M Distilled Water | | | | |
| 0.25 M Sucrose | | | | |
| 0.50 M Sucrose | | | | |
| 0.75 M Sucrose | | | | |
| 1.00 M Sucrose | | | | |

$$\% \text{ Change} = \frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$

TABLE B-2: DIALYSIS BAG RESULTS - CLASS DATA

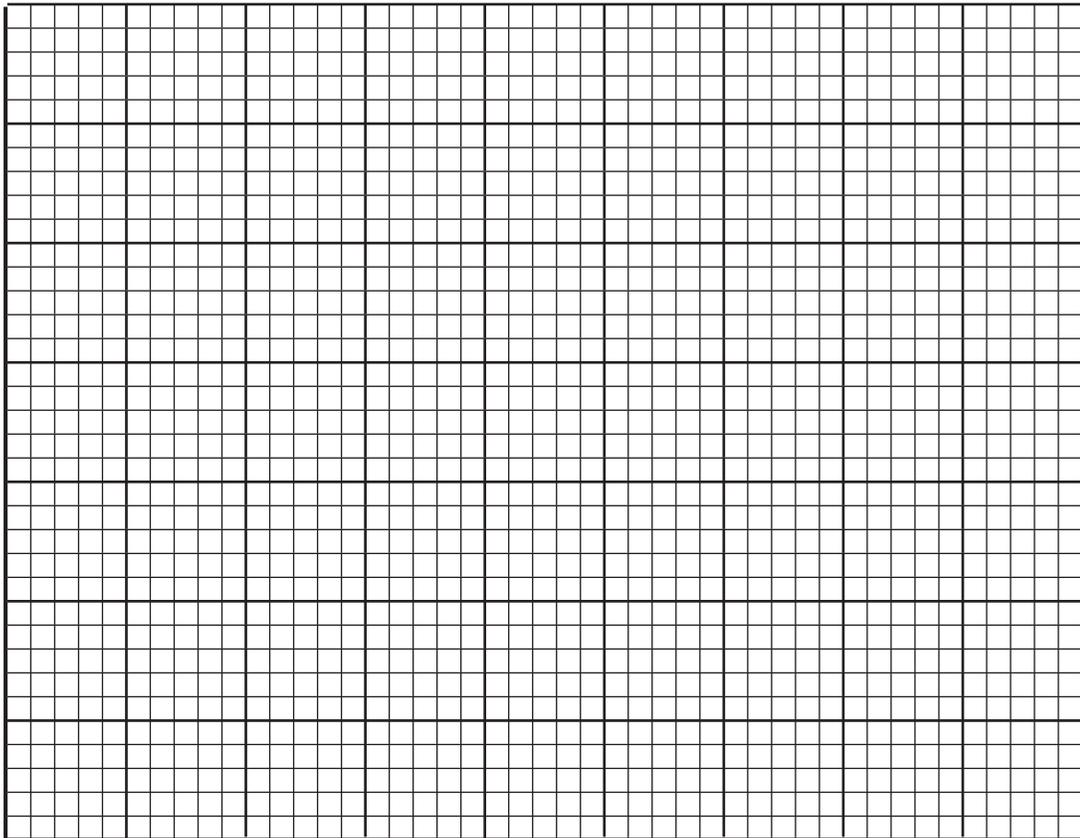
| Molarity of Dialysis Bag | % Change in Mass of Dialysis Bags | | | | | | | | Total | Group Avg. |
|--------------------------|-----------------------------------|----|----|----|----|----|----|----|-------|------------|
| | #1 | #2 | #3 | #4 | #5 | #6 | #7 | #8 | | |
| 0.00 M Distilled Water | | | | | | | | | | |
| 0.25 M Sucrose | | | | | | | | | | |
| 0.50 M Sucrose | | | | | | | | | | |
| 0.75 M Sucrose | | | | | | | | | | |
| 1.00 M Sucrose | | | | | | | | | | |

$$\% \text{ Change} = \frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$



PART B. Analysis of Results: Osmosis

1. Graph the results from both your individual data and the class average.
2. Label the independent variable (horizontal x-axis).
3. Label the dependent variable (vertical y-axis).
4. Title the Graph:



5. What hypothesis is being tested in the experiment?

PART C. Water Potential

In this experiment, the water potential of plant tissue will be determined. The cytoplasm has a lower water potential, Ψ , than pure water because of high concentrations of dissolved solutes (e.g., metabolites, salts, proteins, nucleic acids). Many of these solutes cannot penetrate the membrane and leave the cell by passive diffusion. A lower value of Ψ indicates a lower concentration of water. At some concentration of sucrose in solution, the water potential will equal the water potential of the cell. The chemical nature of the solute does not matter (assuming it is not toxic), since water potential is only determined by the number of solute molecules. Potato cells will be placed into solutions containing different concentrations of sucrose. Some of the solutions will have lower water potentials (higher solute concentration) than the cells and are hypertonic. Others will have higher potentials (lower solute concentration) and are hypotonic relative to the cells. Changes in cell mass will occur by osmosis. Graphical extrapolation of the initial and final masses of the potato cells versus the sucrose concentration will yield the approximate sucrose concentration that is isotonic relative to the cells. This value will be used to calculate the osmotic pressure Ψ_p , which equals Ψ , at constant atmospheric pressure.

1. Obtain a beaker containing a specified sucrose solution or distilled water.
2. Remove any residual skin that may be present on a portion of potato.
3. Cut out three sections that are approximately 3 to 4 cm in length with a cork borer.

Alternatively, cut out three sections that are approximately 0.5 cm wide, 3 to 4 cm long, and 3 - 4 cm deep with a knife.

4. Place the pieces in a beaker or weigh dish. Cover to avoid drying.
5. Determine the mass of the potato sample to the nearest tenth of a gram and record this value (Table C1) as initial mass after obtaining your solution.
6. Immerse the potato sample in the solution and cover the beaker. Let the samples sit overnight.
7. Determine the temperature of the solution in $^{\circ}\text{C}$ and convert to $^{\circ}\text{K}$ by adding 273 to this value. Record in the table under T.
8. Remove the potato samples from the solution and blot dry with a



PART C. Water Potential

paper towel. Keep the sample covered in a beaker or weigh dish to avoid drying.

9. Determine the mass of the sample and record as Final Mass in Table C1.
10. Determine the percent difference between the initial and final masses. Record this value in Table C1 (See Quick Reference, below for calculation formula).

Quick Reference:

$$\% \text{ Change} = \frac{\text{Final Mass} - \text{Initial Mass}}{\text{Initial Mass}} \times 100$$

11. Evenly divide a piece of graph paper by drawing a horizontal line (starting at the midpoint of the Y-axis). Label this line as zero (0) on the Y-axis. This axis will represent the percent change in mass. Alternatively, you may use the graph provided on page 19.
12. Label the Y-axis in increments of negative 5% below the zero (0) point, and in increments of positive 5% above the zero (0) point.
13. Plot the percent change in mass versus the sucrose concentration (molarity) on the X-axis.
14. Draw the best average line through the data points. **The point of intersection (the line determined by the data points and the horizontal line corresponding to 0% change in mass) represents the approximate molarity of sucrose that is isotonic relative to the potato cells.**
15. Calculate Ψ at the isotonic sucrose concentration by determining Ψ_s :

$$\Psi_s = -iCRT$$

The units will be in atmospheres. R = 0.0821 liter x atmosphere/K^o x mol.
C = sucrose molarity.

i = **ionization constant** (since sucrose does not ionize in water, it is 1.0).

C = **Molar concentration** of solute

R = **Pressure constant** (R = 0.0831 liter bars/mole K).

T = **Temperature K** (°C of solution + 273)

PART C. Analysis of Results: Water Potential

TABLE C-1: POTATO CORE RESULTS - INDIVIDUAL DATA

| Molarity in Beaker | Initial Mass | Final Mass | Mass Difference | % Change in Mass | Class Average % Change in Mass |
|--------------------|--------------|------------|-----------------|------------------|--------------------------------|
| 0.0 M | | | | | |
| 0.1 M | | | | | |
| 0.2 M | | | | | |
| 0.3 M | | | | | |
| 0.4 M | | | | | |
| 0.5 M | | | | | |
| 0.6 M | | | | | |
| 0.7 M | | | | | |

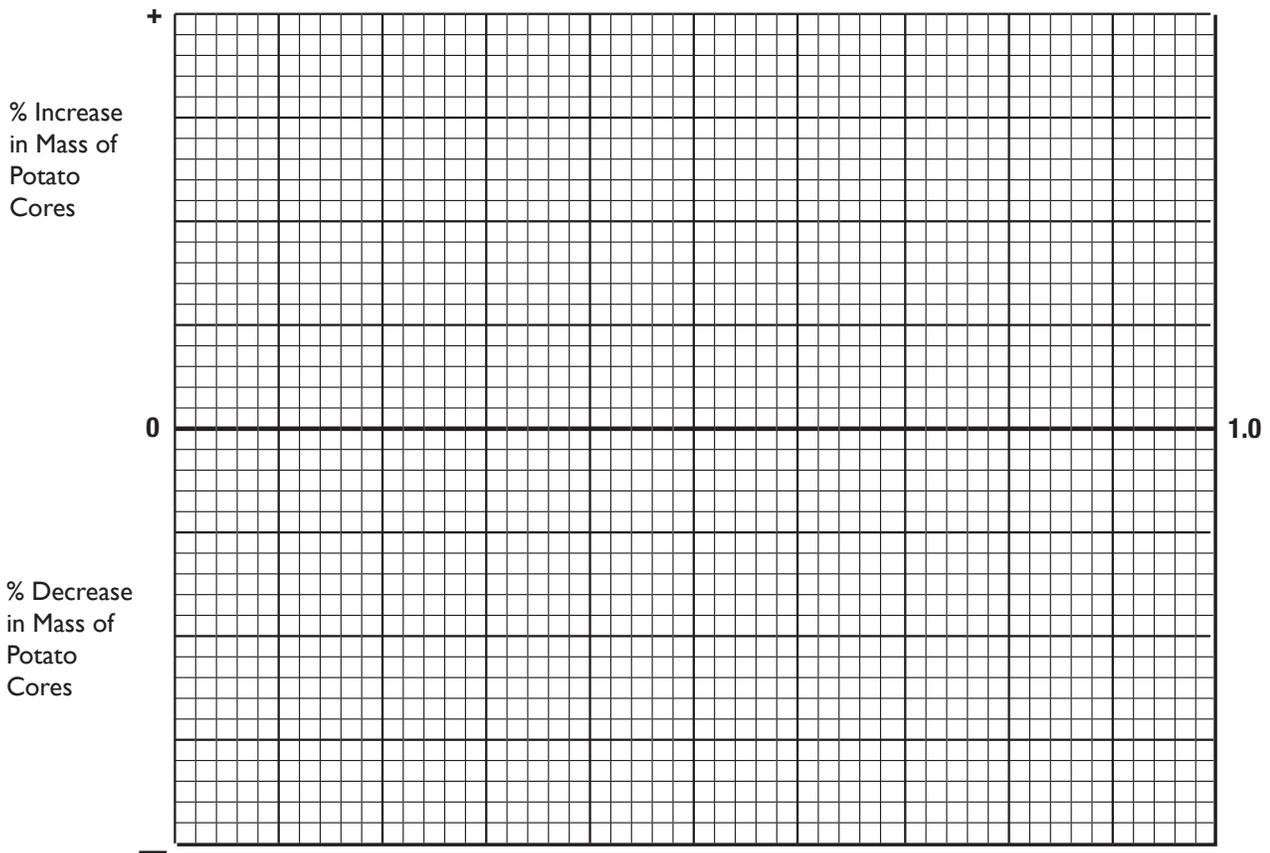
TABLE C-2: POTATO CORE RESULTS - CLASS DATA

| Molarity in Beaker | % Change in Mass of Potato Cores | | | | | | | | Total | Group Avg. |
|--------------------|----------------------------------|----|----|----|----|----|----|----|-------|------------|
| | #1 | #2 | #3 | #4 | #5 | #6 | #7 | #8 | | |
| 0.0 M | | | | | | | | | | |
| 0.1 M | | | | | | | | | | |
| 0.2 M | | | | | | | | | | |
| 0.3 M | | | | | | | | | | |
| 0.4 M | | | | | | | | | | |
| 0.5 M | | | | | | | | | | |
| 0.6 M | | | | | | | | | | |
| 0.7 M | | | | | | | | | | |



PART C. Analysis of Results: Water Potential

1. Graph the results from both your individual data and the class average.
2. Label the independent variable (horizontal x-axis).
3. Label the dependent variable (vertical y-axis).
4. Title the Graph:



5. What is the hypothesis being tested in this experiment?

Experiment Procedure



PART D. Onion Cell Plasmolysis

Prepare a wet mount of epidermal cells of an onion as follows:

1. Place one or two drops of tap water on a microscope slide.
2. Peel off one layer of epidermal cells from an onion and place in the drop of water. Add a cover slip.
3. Observe and draw under 430X magnification. Describe the appearance of the onion cells.
4. Add two or three drops of 12% NaCl to one edge of the cover slip. Place a piece of paper towel along the opposite edge of the coverslip. The tap water will soak into the paper towel, drawing the salt solution under the coverslip.
5. Observe and draw under 430X . Describe what has occurred.



Study Questions

1. Which dye penetrated the membrane in the dialysis experiment? Why?
2. What molecular weight cut off value would allow both dyes to penetrate the membrane?
3. In the idealized case involving non-charged solutes, the equilibrium constant (K_{eq}) for the diffusible dye in the dialysis experiment, is

$$K_{eq} = \frac{[DYE]_{in}}{[DYE]}$$

If K_{eq} is 1, then, at equilibrium, the concentration of dyes on both sides of the membrane are equal, as expected for a passive diffusion experiment. Assuming constant pressure and temperature, answer the following:

- a. Does changing the water bath alter the K_{eq} ?
 - b. Does changing the water bath change the amount of time it takes the system to reach equilibrium?
 - c. Assume the K_{eq} of the diffusible dye in the mixed dye experiment is 10. What could account for a value greater than 1?
4. How could the rate of dialysis of the dye be increased?
 5. How did the mass of the dialysis tubing change with the concentration of sucrose-salt solution? Why? What would you expect if the experiment were reversed, i.e., the bath contained increasing concentrations of sucrose-salt and the tubing initially contained pure water?
 6. A protein having a molecular weight of 65,000 has been purified. The protein solution has a high concentration of sodium chloride as a result of the last purification step. Suggest a method for removing the salt from the protein solution.
 7. Part C. Water Potential, in analyzing the graph obtained by plotting the percent change in mass of potato cores at different molarities of sucrose, what was the approximate molar concentration of sucrose that was isotonic relative to the potato cells?
 8. Calculate the solute potential of the sucrose solution. Refer to experimental background information for formula and values.

Experiment Procedure

Study Questions

- 9. What is the water potential?
- 10. Differentiate among isotonic, hypotonic, and hypertonic solutions in relation to plant and animal cells.
- 11. What is plasmolysis? Explain in terms of the onion cells in the experiment

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Notes to the Instructor

OVERVIEW OF LABORATORY INVESTIGATIONS

The "hands-on" laboratory experience is a very important component of the science courses. Laboratory experiment activities allow students to identify assumptions, use critical and logical thinking, and consider alternative explanations, as well as help apply themes and concepts to biological processes.

EDVOTEK experiments have been designed to provide students the opportunity to learn very important concepts and techniques used by scientists in laboratories conducting biotechnology research. Some of the experimental procedures may have been modified or adapted to minimize equipment requirements and to emphasize safety in the classroom, but do not compromise the educational experience for the student. The experiments have been tested repeatedly to maximize a successful transition from the laboratory to the classroom setting. Furthermore, the experiments allow teachers and students the flexibility to further modify and adapt procedures for laboratory extensions or alternative inquiry-based investigations.

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students. These guidelines can be adapted to fit your specific set of circumstances.

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK web site.

www.edvotek.com

In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800-EDVOTEK (1-800-338-6835).

Safety Data Sheets can be found on our website:

www.edvotek.com/safety-data-sheets



Pre-Lab Preparations

For Part A, each Lab group requires:

- 2 clean beakers (300-400ml) with 250ml distilled water
- Orange & Blue/green dyes in separate test tubes
- 1ml & 5ml pipets
- 1 Empty test tube
- 2 Pieces of hydrated (soaked) dialysis tubing
- Gloves

For Part B, each Lab group requires:

- 5 clean beakers (300-400ml)
- 5 Pieces of hydrated (soaked) dialysis tubing
- 5ml pipets
- Gloves

The class may perform the experiments of Part A and B during the same lab period. Part B should be set up first. Steps 1-10 in Part A can be done during the 30 minute incubation period of Part B.

PART A. DIFFUSION AND DIALYSIS

1. Cut the dialysis tubing into 8 inch sections. For 10 lab groups, Part A will require 20 sections and Part B will require 50.
2. One to two days before the laboratory, soak the dialysis tubing in distilled water.:
 - Place the cut dialysis tubing in a 600ml beaker and cover with 400ml distilled water.
 - Tubing should be covered by distilled water.
3. For each student group:
 - Dispense 1ml of orange indicator dye (A).
 - To a separate test tube, add 2ml of blue/green dye (B).

PART B. OSMOSIS

1. Label 4 clean beakers or flasks (100ml size) distilled water, 0.25 M, 0.5 M, 0.75 M.
2. Prepare dilutions of the 1 M Sucrose - 1 M NaCl solution (experiment component C) in separate beakers:
 - Fill the distilled water vessel with approximately 60ml of distilled water.
 - Add 45ml distilled water to 0.25 M beaker or flask. Add 15 ml of experiment component C (1 M sucrose - 1 M NaCl). Mix.
 - Add 30ml of distilled water to the 0.5 M beaker. Add 30ml of experiment component C. Mix.
 - Add 15ml of distilled water to the 0.75 M beaker. Add 45ml of experiment component C. Mix.
3. Cap the experiment component bottle C and label 1 M.



Pre-Lab Preparations

For Part C, each Lab group requires:

- 1 (or more) beakers of sucrose solution or distilled water
- Ruler
- 3 Pieces of cut potato per group.

PART C. WATER POTENTIAL

1. To a 1 liter graduated beaker:
 - Add all of the powdered sucrose (component D).
 - Add distilled water to the 600ml graduation mark.
 - Mix until the sucrose is dissolved.
 - Adjust to a final volume of 600ml with distilled water. Mix.
 - Label the graduated cylinder as 1M Sucrose.
 - There will be enough 1 M sucrose solution to do the water potential experiment two times. It must be sterilized for storage periods longer than 2 days.
2. On the day of the lab, prepare the sucrose solutions in separate beakers and mix as follows:
 - 90ml distilled water and 10ml 1 M sucrose. Label 0.1 M.
 - 80ml distilled water and 20ml 1 M sucrose. Label 0.2 M.
 - 70ml distilled water and 30ml 1 M sucrose. Label 0.3 M.
 - 60ml distilled water and 40ml 1 M sucrose. Label 0.4 M.
 - 50ml distilled water and 50ml 1 M sucrose. Label 0.5 M.
 - 40ml distilled water and 60ml 1 M sucrose. Label 0.6 M.
 - 30ml distilled water and 70ml 1 M sucrose. Label 0.7 M.
 - 100ml distilled water. Label distilled water.

(You may wish to make a second set of these solutions to obtain averages.)

3. Remove the skin from 4 large potatoes and keep them covered in the refrigerator.

PART D. ONION CELL PLASMOLYSIS

1. On day of the lab, prepare the salt solution as follows:
 - Dissolve 12 g of salt in 100ml of water. Mix and label "12% NaCl".
2. Students can use this stock solution for Part D.

Experiment Results and Analysis

Part A. The water bath around the dialysis bag containing the mixture of dyes will become orange/green. The solution inside the bag will eventually become blue (after three 250ml bath changes over a period of approximately 15 hours). There should be no changes with the system that contains only the blue dye.

Part B. The mass of the bags increase with increasing concentrations of sucrose-salt. Representative results are shown below.

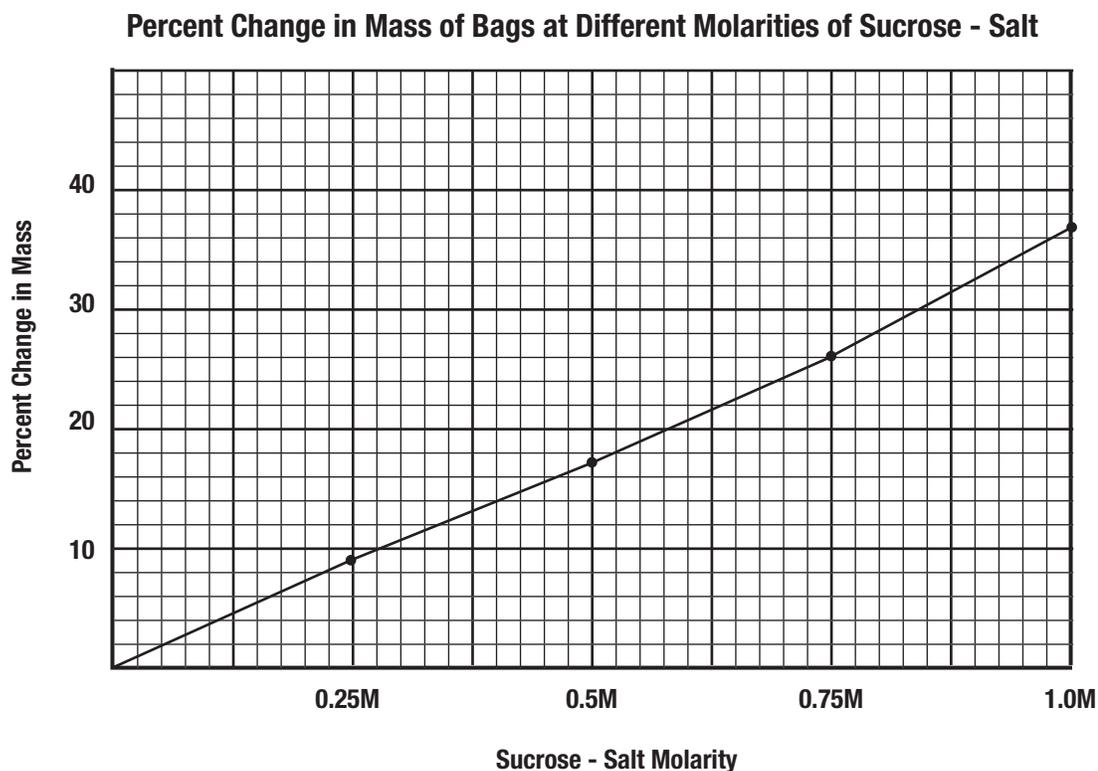
| MOLARITY | % CHANGE |
|----------|----------|
| 0 | 0 |
| 0.25 | 9 |
| 0.5 | 17 |
| 0.75 | 26 |
| 1.0 | 37 |

Part C. Experimentally determined Y values under the conditions of this experiment are in the range of -5.0 to -8.0.

Part D. The salt solution added to the onion cells, will result in water leaving the cells and the central vacuole will shrink and the cytoplasm will shrink.



Experiment Results and Analysis



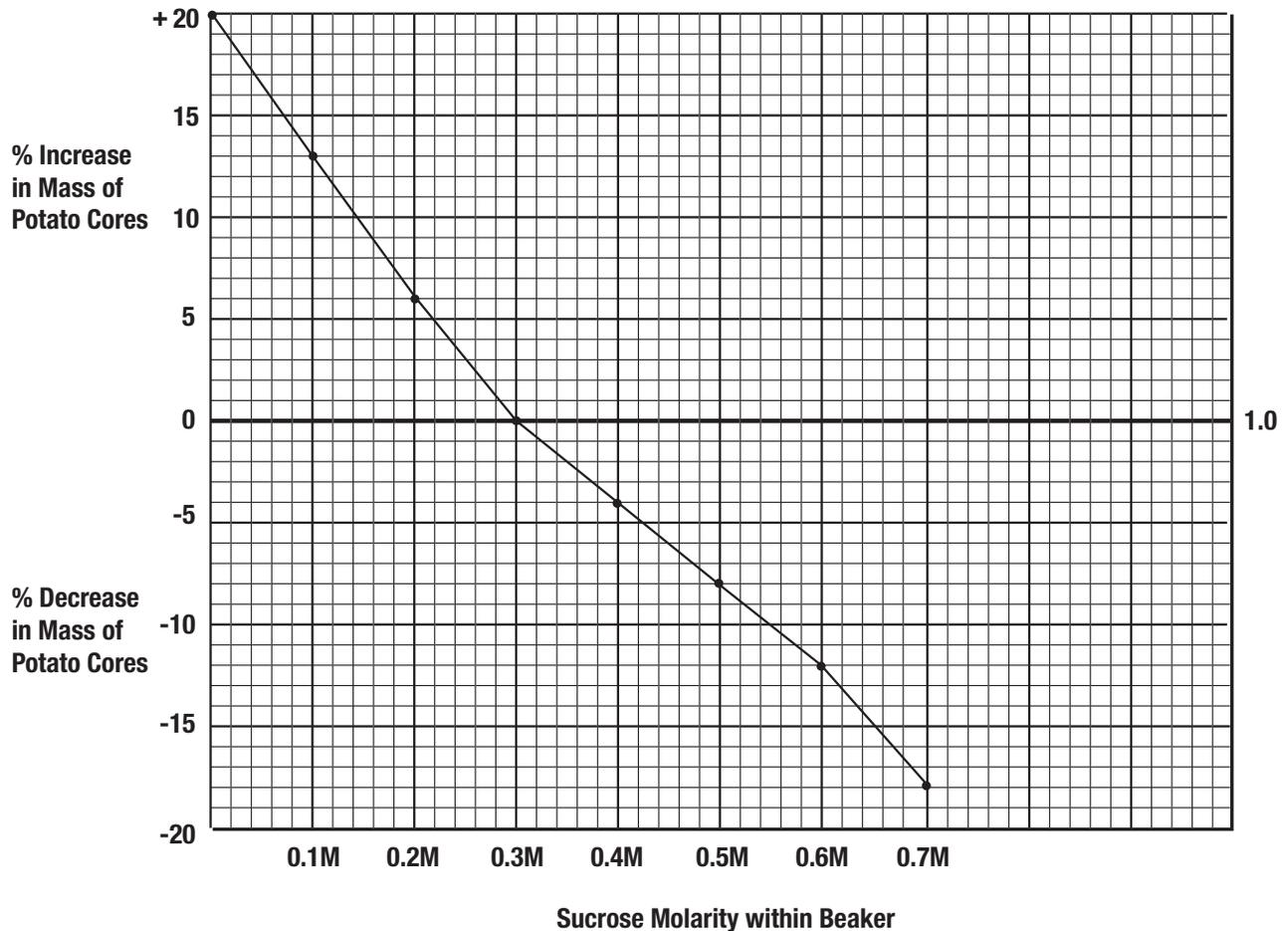
Label the independent variable (horizontal x-axis):
Sucrose - Salt Molarity

Label the dependent variable (vertical y-axis):
Percent Change in Mass

Title the Graph:
Percent Change in Mass of Bags at Different Molarities of Sucrose - Salt

What hypothesis is being tested in this experiment?
If dialysis tubing is filled with different molarities of sucrose-salt solution, then water will move into the bag in relation to molarity when placed into a distilled water environment.

Experiment Results and Analysis - Part C: Water Potential



Label the independent variable (horizontal x-axis):
Sucrose Molarity within the Beaker

Label the dependent variable (vertical y-axis):
Percent Change in Mass (+/-) of Potato Cores

Title the Graph:
Percent Change in Mass of Potato Cores at Different Molarities of Sucrose

What hypothesis is being tested in this experiment?
If water potential is affected by solute concentration, then adding solute to a solution will cause the water potential to become more negative.



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Study Questions and Answers

1. Which dye penetrated the membrane in the dialysis experiment? Why?

The orange & green dyes diffused out of the tubing because their molecular weights are lower than the cut off of the membrane. In other words, the orange & green dyes are small enough to pass through the pores. The chemical potential of the dyes are initially higher inside the tubing, consequently it diffuses to a region of lower concentration (outside).

2. What molecular weight cut off value would allow both dyes to penetrate the membrane?

100,000 or less.

3. a. Does changing the water bath alter the K_{eq} ?

No. K_{eq} is a constant at a given temperature and pressure. Its value is independent of the concentration of dyes.

- b. Does changing the water bath change the amount of time it takes the system to reach equilibrium?

Yes. It will shorten the time.

- c. Assume the K_{eq} of the diffusible dye in the mixed dye experiment is 10. What could account for a value greater than 1?

If the orange dye interacted with the non-diffusing blue/green dye the K_{eq} would be larger and would represent an equilibrium binding constant.

4. How could the rate of dialysis of the dye be increased?

Increasing the temperature increases the rate of dialysis because higher temperatures increase molecular motion. Constant stirring of the bath also increases the rate because it distributes the dye through the solution more rapidly.

5. How did the mass of the dialysis tubing change with the concentration of sucrose-salt solution? Why? What would you expect if the experiment were reversed, i.e., the bath contained increasing concentrations of sucrose-salt and the tubing initially contained pure water?

The mass of the tubing contents increases with increasing solute concentration (positive slope on the graph) because of a net influx of water. Increasing solute concentration decreases the water concentration, therefore more water flows into the tubing. This is osmosis. If the experiment were reversed, the mass of the tubing contents would decrease with increasing concentration of bath solutes.

6. A protein having a molecular weight of 65,000 has been purified. The protein solution has a high concentration of sodium chloride as a result of the last purification step. Suggest a method for removing the salt from the protein solution.

Dialysis with membrane having a cut off that is less than 65,000.

**Please refer to the kit
insert for the Answers to
Study Questions**